Acute Lymphoblastic Leukemia of Burkitt’s Type (L-3 ALL)
Lacking Surface Immunoglobulin and the 8;14 Translocation

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A 25-year-old man developed acute lymphoblastic leukemia, morphologically of Burkitt’s type (L3-ALL, FAB classification) but with immunologic and cytogenetic features not previously reported. The leukemic blasts were B1+, CALLA+, OKT3−, and OKT11−. Surface immunoglobulin and cytoplasmic IgM were not detected, but cytoplasmic IgG lambda was present. Karyotypic analysis of 20 metaphases was normal at presentation but abnormal after relapse. At that time, the predominant karyotype was 47XY, lq−, 7q−, 12p−, M1. This was present. Karyotypic analysis of 20 metaphases was normal

Received December 20, 1983; received revised manuscript and accepted for publication February 28, 1984.

Supported in part by BRSG S07-RR05394 awarded by the Biomedical Research Support Grant Program. Division of Research Resources National Institute of Health and by the Veterans Administration. Address reprint requests to Dr. Mangan: Hematology Division, Montefiore Hospital, 3459 Fifth Avenue, Pittsburgh, Pennsylvania 15213.

22 may not be found in all cases of L-3 ALL. (Key words: Burkitt’s leukemia; Pre-B-cell acute lymphoblastic leukemia) Am J Clin Pathol 1985; 83: 121–126

THE WIDELY ACCEPTED French–American–British (FAB) classification of the acute leukemias recognizes a rare lymphoblastic variant termed L-3 ALL, which is morphologically indistinguishable from the classic Burkitt lymphoma cell.2 The blasts readily are identified by numerous lipid laden vacuoles in an intensely basophilic cytoplasm. Clinically, the disease is aggressive and often fatal within six months.9 In most cases studied, the
leukemic blasts bear surface immunoglobulin\(^2,3,9\) and cytogenetically have a translocation between chromosomes No. 8 and 14, \((t\ 8;14))\.2,3,9,29 Nonrandom variant translocations involving chromosomes No. 8 and 22, \((t\ 8;22)) or No. 2, \((t\ 2;8)) have also been reported.3 Although the immunologic and cytogenetic features of L-3 ALL are remarkably constant,2,3,9,12,29 rare exceptions have been reported.3,5,8,10,14,22,29 We describe here an additional case of L-3 ALL with immunologic and cytogenetic features not previously reported.

**Report of a Case**

A 25-year-old white man presented to the Albany Medical Center Hospital with fever and bone pain in the left hip and lumbosacral spine. The physical examination revealed no lymphadenopathy or hepatosplenomegaly or masses. A blood count revealed the following: hemoglobin 15 g/dl, hematocrit 42.8%, platelets 63,000/mm\(^3\), and WBC 10,700/mm\(^3\). The differential count revealed 43% segmented neutrophils, 27% bands, 16% lymphocytes, 11% monocytes, and 3% lymphoblasts with deep basophilic cytoplasm, containing prominent vacuoles ringing the nucleus. A bone marrow aspirate and biopsy were packed with >90% blasts resembling Burkitt cells (Fig. 1). Additional studies were performed on the marrow blasts to document the nature of the leukemia (see "Special Studies"). Light and electron microscopic examination of a left axillary lymph node revealed reactive hyperplasia without Burkitt tumor cells. The serum lactate dehydrogenase (LDH) was 589 U/mL (normal < 200) and later rose to >2,000 U/mL. Antibody titers to Epstein-Barr virus capsid antigen\(^1\) were 1:10, 1:25, 1:50 at presentation, two months, and four months later, respectively, consistent with previous infection at an undetermined time. A skeletal survey revealed no tumor masses or lytic lesions. Liver-spleen scan, abdominal gallium scan, lymphangiogram, and echograms of the heart, retroperitoneum, and pelvis were all negative for tumor masses or enlarged lymph nodes. A venous pyelogram, and a thoracic spinal fluid was negative. A clinical diagnosis of acute lymphocytic leukemia (lymphoblastic) was made. A bone scan showed abnormal uptake in the parietal skull and 5th and 7th ribs. Cytology of spinal fluid was negative. A blood count revealed the following: WBC 10,700/mm\(^3\). The differential count revealed 43% segmented neutrophils, 27% bands, 16% lymphocytes, 11% monocytes, and 3% lymphoblasts in the peripheral blood smear. A blood count revealed the following: hemoglobin 15 g/dl, hematocrit 42.8%, platelets 63,000/mm\(^3\), and WBC 10,700/mm\(^3\). The differential count revealed 43% segmented neutrophils, 27% bands, 16% lymphocytes, 11% monocytes, and 3% lymphoblasts in the peripheral blood smear.

**Special Studies**

**Cytochemical Studies**

Smears were prepared from marrow aspirated at presentation (>90% leukemic blasts). Wright–Giemsa, methyl green pyronin (MGP), and oil red 0 stains were done as described.16 Wright–Giemsa stained blasts (Fig. 1) demonstrated the characteristic cytoplasmic vacuoles and intense basophilia of Burkitt leukemia cells. The cytoplasm was MGP positive, and the cytoplasmic vacuoles were oil red 0 positive. Peroxidase, periodic acid-Schiff (PAS), alpha naphth-acetate esterase with and without fluoride inhibition, acid phosphatase, and beta glucuronidase stains (kindly performed by Dr. John Bennett, University of Rochester, School of Medicine) all had negative results.

**Ultrastructural Studies**

Marrow particles were fixed in 3.8% paraformaldehyde in Millonig's buffer, overlaid in 2% agar, postfixed in 2% osmium tetroxide, and embedded in Epon 812. Sections were cut on an LKB ultratome 3, doubly stained with uranyl acetate and lead citrate, and viewed with a Phillips 300\(^\text{a}\) transmission electron microscope. A representative leukemic blast (Fig. 2) demonstrates lipid laden vacuoles, prominent ribosomes, and absence of primary granules consistent with the lymphoblastic nature of the cells.

**Immunologic Typing Studies**

Blast cell suspensions were prepared by Ficoll–Hypaque\(^\text{a}\) density gradient centrifugation,\(^14\) then washed three times to remove unbound proteins. Surface immunoglobulins (slg) and cytoplasmic immunoglobulins (clg) were detected as described\(^1\) by direct immunofluorescence employing monospecific (Fab\(_\text{a}\)) fragments of fluorescein-conjugated anti-human heavy chain (IgG, IgA, IgM, IgD) and light chains (kappa, lambda) Meloy Labs, Springfield, Virginia). A minimum of 200 leukemic blasts were scored for reactivity employing a Zeiss\(^\text{a}\) immunofluorescence microscope. Controls were always less than 5% positive. Slg was not detected employing monospecific FITC conjugated antibodies to either heavy or light chains. However, 53% and 51% of leukemic blasts demonstrated intracytoplasmic IgG (Fig. 3) and lambda, respectively. Intracytoplasmic IgM, IgA, IgD, or Kappa chains were not detected. An indirect immunofluorescent technic as described by the manufacturer was employed to detect the common acute lymphoblastic leukemia antigen (CALLA [J-5], Coulter Electronics, Hialeah, FL), a pan B-cell antigen (B1, Coulter Electronics), an E-rosette receptor antigen (OKT11, Ortho Pharmaceuticals, Raritan, NJ), and a pan T-cell antigen (OKT3, Ortho Pharmaceuticals). Labeled cells were analyzed by a fluorescent activated cell sorter (FACS IV, Becton–Dickinson). Controls for the FITC-labeled goat anti-mouse IgG antibodies (Cappel Labs, Cochranville, PA) were always <5% positive. 85% and 81% of the leukemic blasts reacted with the B1 and CALLA antigen.
Fig. 1 (upper). Wright-Giemsa stained marrow (×1,600) illustrating large basophilic blasts containing prominent cytoplasmic vacuoles.

Fig. 2 (lower). Electron micrograph (×16,200) of a Burkitt cell leukemic blast illustrating prominent lipid vacuoles (L), scattered mitochondria (m), increased ribosomes (r) with endoplasmic reticulum (er), golgi apparatus (g), and reduplicated nuclear membrane (nm). Primary granules are absent in the cytoplasm.
FIG. 3 (upper). Immunofluorescent photomicrograph of leukemic blasts (magnification X2,500) showing staining of intracytoplasmic IgG heavy chains in approximately one-half of the marrow cells. Vacuoles can be discerned in the cytoplasm of several blasts that react with the label. Intracytoplasmic lambda light chains also were detected, but cytoplasmic IgM, IgD, IgA, or kappa chains were absent.

FIG. 4 (lower). Composite karyotype illustrating the constant chromosomal anomalies detected at relapse. One line was 47,XY,1q-, 7q-, 12p-. Mar 1. A second line had the above abnormalities but possessed 48 chromosomes due to presence of Mar 2. While Mar 1 resembled the long arms of chromosome 1, it was larger and had a centromere. Mar 2 could not be identified and also had a centromere. Also present were several metaphases with 47 chromosomes but possessing both markers: these appeared to be representatives of the 48-chromosome line that had sustained random loss of chromosomes.
antibodies, respectively. Only 5% and 9% of the blasts reacted with the T-cell antibodies OKT3 and OKT11, respectively. SIg studies and monoclonal antibody studies were repeated after second relapse and gave similar results. In summary, the leukemic blasts were CALLA+, B1+, SIg−, c IgG+, c IgM−, OKT3−, OKT11−.

**Cytogenetics**

Chromosome analyses were performed by direct techniques and after 24 hours in culture on marrow specimens replaced with (>90%) blasts employing a trypsin–Giemsa binding technic.23 No chromosomal abnormalities were detected in 20 metaphases from marrow cell suspension studied at presentation before treatment. After relapse, cytogenetic studies of bone marrow were repeated and 28 metaphases were examined (Fig. 4). The chromosome numbers were 45 (5 cells), 46 (2), 47 (20) and 48 (1). The two cells with 46 chromosomes were the only normal cells present. The cell with 48 chromosomes possessed the karyotype 48 XY 1q− 7q− 12p− with two marker chromosomes. M1 resembled the long arm of chromosome No. 1 but was larger and possessed a centromere (Fig. 4). M2 could not be identified but also possessed a centromere. The remaining 25 cells all possessed M1, and 13 of them also possessed M2. The cells with 45 chromosomes, and those with 47 chromosomes but possessing both M1 and M2, all showed apparently random losses of other chromosomes. Thus, there appeared to be two distinct but related cell lines: 47 XY 1q− 7q− 12p− M1 and 48 XY 1q− 7q− 12p− M1 M2. The cells with 47 chromosomes and both markers appeared to represent members of the 48-chromosome line that had sustained random chromosome loss and not another cell lineage. A careful search did not disclose any abnormalities of chromosomes 2, 8, 14, or 22.

**Discussion**

The leukemic blasts in L-3 ALL usually display surface immunoglobulin (SIg) and a translocation between chromosomes 8 and 14, (t 8;14).3,4,8,13,29 Because these features are also found in the morphologically identical Burkitt’s lymphoma cells (BL),17 it has been suggested that these malignancies are different manifestations of the same disease, involving a lymphocyte committed to the B-cell lineage.3 However, exceptions to these findings have been noted. A variety of SIg–lymphomas potentially may masquerade as L-3 ALL if blood and marrow invasion occurs.17 In addition, three cases of an acute nonlymphocytic leukemia (ANLL), one with erythroleukemia8 and two with acute myelomonocytic leukemia, have been reported with L-3 morphology.5 There is even one case of metastatic carcinoma with this morphology.2 Rarely a T-cell ALL or null cell ALL has been described with L-3 morphology.5,10,14,19,29 In only one of these cases was intracytoplasmic immunoglobulin examined and IgM found.10 To our knowledge, the present case is the first report of an L-3 ALL lacking surface immunoglobulin and T-cell markers but with intracytoplasmic IgG. These cases underscore the need for extensive immunologic, histochemical, and ultrastructural studies in L-3 ALL before a definitive diagnosis can be made.

Despite the absence of SIg on our patient’s blasts, the B-cell lineage of the leukemia clearly was documented by the expression of the B1 antigen and intracytoplasmic IgG. The B1 antigen is expressed in all B-cell differentiation stages except the plasma cell.23 The absence of SIg and the presence of intracytoplasmic IgG suggests that a leukemogenic event may have occurred in a pre-B-cell. Ganick and Finlay previously have reported one case of L-3 ALL lacking surface immunoglobulin but with intracytoplasmic IgM.10 Although the presence of intracytoplasmic IgM and absent surface immunoglobulin usually defines a pre-B-cell,6 Vogler has noted a small portion of leukemic blasts that contain detectable cytoplasmic IgG or IgA.30 The present case may represent an expansion of this uncommon pre-B-cell phenotype.

In two recent reviews of cytogenetic findings in L-3 ALL,3,29 almost all cases had abnormalities involving chromosomes 14, 2 or 22, the location of genes encoding heavy and light chain immunoglobulin synthesis.12,13,18,20 The most common abnormalities were t 8;14 or variants: 14 q+, t 8;22, or t 2;8.3,29 Only five cases of L-3 ALL have been reported lacking involvement of chromosomes 8 or 14: one patient had t 4;11,28 another 6q−,26 and a third, an abnormality of chromosome 1.27 In addition to our patient, only two other patients with L-3 ALL have been reported with normal chromosomes.7,29 A single patient, reported by the International Workshop,29 with cells lacking surface immunoglobulin and the t 8;14, resembles our case; intracytoplasmic immunoglobulin studies were not done. It seems unlikely that nonleukemic cells were responsible for the normal karyotype observed in our case at presentation since studies were done directly and after 24 hours in culture from marrow packed with leukemic blasts. Furthermore, we did not see an evolution of a (t 8;14) clone or related abnormality over a 33-month period. When the karyotype was studied late in the course of the disease, our patient had a 7q− aberration and a marker chromosome resembling the long arm of chromosome 1 but not identical with it. These abnormalities have been noted previously in patients with ALL and lymphoma.15,24,27 Despite the hematologic and cytogenetic alterations, the Burkitt cell morphology persisted. Barring the possibility of a submicroscopic or point mutation in chromosomes
14, 2, or 22, our case illustrates that rearrangements in these chromosomes involved in immunoglobulin synthesis may not be required in all instances for evolution of a lymphoblastic leukemia with Burkitt morphology.

Acknowledgments. The authors are grateful to John Bennett, M.D., University of Rochester, School of Medicine, Rochester, NY, for preparation and review of the cytochemical studies, to Dora Darcangelis, New York State Virology Lab for the EB virus serology studies, and to Mary T. Almade and Joan Salustri for outstanding secretarial assistance.

References